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Abieticola koreana gen. et sp. nov., a griseofulvin-producing endophytic xylariaceous ascomycete from Korea

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Abstract—A new genus and species. *Abieticola koreana,* is described. This xylariaceous fungus was isolated from the inner bark of a Manchurian fir (*Abies holophylla*) in Korea. Phylogenetic analyses based on the sequences of four gene regions—ITS1-5.8S-ITS2, 28S, β-tubulin, and rpb2—were used to confirm this new genus and species.

Key words—*Ascomycota*, anamorph, multigene, Xylarioideae, *Xylariaceae*

Introduction

The *Xylariaceae* is a large family of ascomycetes comprising approximately 85 genera and at least 1,340 species that are distributed worldwide and exhibit exceptional diversity in the tropics (Whalley 1996, Velmurugan et al. 2013, Stadler et al. 2014). It has been estimated that the *Xylariaceae* contains 10,000 undescribed species (Stadler 2011, Richardson et al. 2014). Recent phylogenetic studies (Tang et al. 2009, Daranagama et al. 2015) using combined ITS, LSU, rpb2, and β-tubulin sequences, suggested that *Xylariaceae* has two major lineages, Xylarioideae and Hypoxyloideae.

To date, 11 genera of *Xylariaceae* have been shown to occur as endophytes of various plants (Pažoutová et al. 2013), including firs, palms, orchids, bromeliads, aroids, ferns, and rainforest trees (Dreyfuss & Petrini 1984, Bayman

et al. 1998; San Martín et al. 2001; Park et al. 2005). Recently, endophytes have been predominantly investigated because of their ability to produce either new or interesting secondary metabolites and for their potential use in bioenergy applications (Bayman et al. 1997, Isaka et al. 2000, Boonphong et al. 2001, Wiyakrutta et al. 2004, Park et al. 2005, Liu et al. 2007).

During screening for organisms that produce antifungal agents, an endophytic fungus designated as F0010, was isolated from the inner bark of *Abies holophylla* (Manchurian fir), a tree that is widespread in Korea (Park et al. 2005). Initially, the isolate was provisionally identified as an unknown species of *Xylaria* and was shown to produce two antifungal metabolites, dechlorogriseofulvin and griseofulvin (Park et al. 2005). However, further investigation suggested that F0010 differs from species classified in *Xylaria* based on both morphological details of the anamorph and on molecular phylogenetic analyses.

The objectives of this study were to image the ultrastructure of the conidial state of F0010 by scanning electron microscopy (SEM), for comparison of its mode of conidiogenesis with that of other *Xylariaceae*, and to investigate its phylogenetic relationship to other xylariaceous fungi based on a multigene analysis, including the nuclear ribosomal ITS1-5.8S-ITS2 and 28S subunit, rpb2, and β-tubulin gene sequences. As a result of this study, a new genus *Abieticola* and species *A. koreana* are proposed here to accommodate this novel xylariaceous fungus.

Materials & methods

Fungal isolation

Samples of *Abies holophylla* bark were collected from a garden in Daejeon, Korea, placed in zip-lock bags, stored in a refrigerator, and then used for the isolation of endophytic fungi within 72 h after sampling. Samples were cleaned under running tap water and then air-dried. The samples were surface sterilised by immersion in 70% ethanol for 1 min and a 3.5% sodium hypochlorite solution for 5 min. Next, the surface-sterilized samples were washed in sterile water three times to remove the surface sterilization agents (Wiyakrutta et al. 2004). The samples were then cut into small pieces and placed on cornmeal malt extract agar (17.0 g of cornmeal, 20.0 g of malt extract, 2.0 g of yeast extract, and 20 g agar in 1.0 L of distilled water) supplemented with chloramphenicol (50 mg/mL) (Sigma, St. Louis, MO, USA), and then incubated at 25°C for up to 3 weeks. Individual fungal strains were transferred to potato dextrose agar (PDA, Bacto Potato Dextrose Dehydrated, Becton Dickinson, USA) and incubated at 25°C for at least 2 weeks. After checking for purity, each fungal culture was transferred to a fresh agar plate. Stock cultures of test species were grown on MEA at 25°C and used as inocula. The basal medium consisted of 2% malt extract agar (MEA, Difco) at pH 5.5. The water activity (a_w) of the basal MEA medium was 0.999, which was modified to 0.95, 0.98, and 0.995 by addition of KCl and glucose. The a_w of media was measured using

a Novasina ICII (Novasina AG, Zurich, Switzerland). The growth of the fungus was determined by transferring mycelial plugs (5 mm diameter) from the growing margin of stock cultures in Petri dishes (90 × 15 mm). After inoculation, plates with the same a_w were sealed in polyethylene bags and incubated at 20, 25, and 30°C in darkness for up to 10 days. Mycelial growth was recorded periodically by measuring the diameter of the colony in two directions at right angles to each other.

Ten strains, F0010-1 to F0010-10, were derived from isolate F0010. Dried MEA cultures were conserved in Chonnam National University Fungal Collection, Gwangju, Korea (CNUFC), and live cultures were conserved in CNUFC and in the Korean Collection for Type Cultures, Daejeon, Korea (KCTC).

Ultrastructure of isolate F0010

The micromorphological features of strain F0010-1 were investigated by light microscopy (LM) with a Nikon Labophot 2 Microscope (Nikon, Tokyo, Japan) with differential interference contrast optics. For scanning electron microscopy (SEM), samples were fixed in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h, post-fixed with 1% OsO_4 in the same buffer for 1 h, dehydrated in graded ethanol, substituted by isoamyl acetate and then critical-point dried in CO_2. Finally, the sample was sputter-coated with gold (SC502; Polaron, West Sussex, UK) and observed with a SEM515 scanning electron microscope (Phillips, Eindhoven, the Netherlands) and an S-3500N low vacuum scanning microscope (Hitachi Science Systems Ltd., Tokyo, Japan).

DNA extraction and PCR amplification

Strains F0010-1 and F0010-2 were grown on PDA, covered with cellophane at 25°C for 4–5 days. Genomic DNA was extracted using the HiGene™ Genomic DNA Prep Kit for fungi (Biofact Co., Daejeon, Korea). The target regions, ribosomal ITS1-5.8S-ITS2, 28S subunit, β-tubulin, and rpb2 (RNA polymerase II subunit 2) were amplified by PCR (Table 1) in 20 µL reaction mixtures containing 2 µL of genomic DNA, 1.5 µL of each primer (5 pM), 14 µL of demineralised sterile water, and 1 µL of PCR premix

Table 1. Primers and annealing conditions used in the molecular analysis.

Locus	Primer	Sequence (5′–3′)	Annealing temp. (°C)	Reference
28S rDNA	LR5F	GCTATCCTGAGGGAAAC	55 (30 sec)	Vilgalys & Hester 1990
	LR0R	ACCCGCTGAACTTAAGC		
β-tubulin	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	58 (1 min)	Sang et al. 2010
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC		
ITS rDNA	ITS1	TCCGTAGGTGAACCTGCGG	55 (30 sec)	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC		
rpb2	RPB2-5F_Eur	GAYGAYCGKGAYCAYTTC	50–72 (1 min), ramping 0.3°C per sec	Hibbett 2006; Houbraken et al. 2011
	RPB2-7CR_Eur	CCCATRGCYTGYTTRCCCAT		

(Bioneer, Daejeon, Korea) using the following fungal-specific primer sets: ITS1 and ITS4 for ITS1-5.8S-ITS2, LROR and LR5F for 28S (White et al. 1990), Bt2a and Bt2b for β-tubulin (Sang et al. 2010), and RPB2-5F-Eur and RPB2-7cr-Eur for RPB2 (Hibbett 2006, Houbraken et al. 2011).

DNA sequencing and phylogenetic analyses

Amplified PCR products of strains F0010-1 and F0010-2 were electrophoresed on a 0.75% agarose gel. The amplicons were purified with the AccuPrep PCR Purification Kit (Bioneer). The purified PCR products were sequenced using an ABI 3700 automated DNA sequencer (Applied Biosystems Inc., USA). The four gene sequences generated were initially aligned with other sequences from GenBank using Clustal X v. 2.0 (Larkin et al. 2007) with a gap opening penalty of 10.0 and a gap extension penalty of 0.05. BioEdit v. 7.2.5 (Hall 1999) was used to manually exclude ambiguous and uninformative variable sites. The alignment was manually refined using MEGA v. 6.0 (Tamura et al. 2013). The ITS, 28S, β-tubulin, and rpb2 gene sequences of F0010-1 and F0010-2 were deposited in GenBank (see TABLE 2).

TABLE 2. Sequences used in the phylogenetic multigene analysis.

TAXON	SAMPLE	GENBANK ACCESSION NO.			
		ITS	LSU	rpb2	β-tubulin
Abieticola koreana **(T)**	**EML-F0010-1**	**JN977612**	**JQ014618**	**KP792128**	**KP792126**
A. koreana	**EML-F0010-2**	**KP835547**	**KP835546**	**KP792129**	**KP792127**
Annulohypoxylon moriforme var. *microdiscum*	CBS123834	DQ631935	DQ840061	DQ631960	DQ840095
A. stygium	MFLUCC 12-0826	KJ940870	KJ940869	KJ940868	KJ940867
Anthostomella brabeji	CBS 110128	EU552098	EU552098	—	—
A. proteae	CBS 110127	EU552101	EU552101	—	—
Astrocystis concavispora	MFLUCC 14-0174	KP297404	KP340545	KP340532	KP406615
A. mirabilis	HAST 94070803	GU322448	—	GQ844835	GQ495941
A. sublimbata	HAST 89032207	GU322447	—	GQ844834	GQ495940
Collodiscula japonica	CBS 124266	JF440974	—	—	JF440974
Daldinia concentrica	CBS 113277	AY616683	—	KC977274	—
D. decipiens	CBS 122879	JX658441	—	—	AY951694
D. loculata	BCRC 34117	EF026145	—	AY951698	—
Discoxylaria myrmecophila	JDR 169	GU322433	—	GQ844819	GQ487710
Entoleuca mammata	JDR 100	GU300072	—	GQ844782	GQ470230
Euepixylon sphaeriostomum	JDR 261	GU292821	—	GQ844774	GQ470224
Hypoxylon fendleri	MFLUCC 12-0816	KM017563	KM017565	KM017566	KM017564

H. fragiforme	MUCL 51264	KM186294	KM186295	KM186296	KM186293
H. lenormandii	MFLUCC 13-0120	KM039135	KM039136	KM039137	KM039138
H. monticulosum	MFLUCC 12-0818	KM052716	KM052717	KM052719	KM052718
Kretzschmaria guyanensis	HAST 89062903	GU300079	—	GQ844792	GQ478214
Lunatiannulus irregularis	MFLUCC 14-0014	KP297398	KP340540	KP340526	KP406609
Nemania diffusa	FR AT-113	DQ658238	DQ840073	DG631947	DQ840088,
N. serpens	FR AT-114	DQ631942	DQ840075	DQ631948	DQ840086
Podosordaria mexicana	WSP 176	GU324762	—	GQ853039	GQ844840
P. muli	WSP 167	GU324761	—	GQ853038	GQ844839
Poronia pileiformis	WSP 88113001	GU324760	—	GQ853037	GQ502720
Rhopalostroma lekae	MFLUCC 13-0123	KJ472428	KJ472427	KJ472429	—
Rosellinia lamprostoma	HAST 89112602	EF026118	—	GQ844778	EF025604
R. merrillii	HAST 89112601	GU300071	—	GQ844781	GQ470229
R. necatrix	HAST 89062904	EF026117	—	GQ844779	EF025603
R. sancta-cruciana	HAST 89062903	GU292824	—	GQ844777	GQ470227
Rostrohypoxylon terebratum	CBS 119137	DQ631943	DQ840069	DQ631954	DQ840097
Ruwenzoria pseudoannulata	MUCL 51394	GU053568	—	—	—
Stilbohypoxylon quisquiliarum	YMJ 89091608	EF026120	—	GQ853021	EF025606
Thamnomyces camerunensis	MUCL 51396	FN428828	—	—	—
Xylaria adscendens	JDR 865	GU322432	—	GQ844818	GQ487709
X. bambusicola	WSP 205	EF026123	—	GQ844802	AY951762
X. hypoxylon	CBS 122620	AM993141	KM186301	KM186302	KM186300

CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, BCRC Bioresource Collection and Research Centre, Taiwan, HAST Herbarium, Research Centre for Biodiversity, Academia Sinica, Taipei, JDR Herbarium of Jack D. Rogers, MFLCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, MUCL Mycotheque de l'Université Catholique de Louvain, Germany, YMJ Herbarium of Yu Ming Ju, WSP Washington State University, USA, EML Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, South Korea; T ex-holotype strain.

Maximum-likelihood (ML) (Felsenstein 1985) algorithms were applied using the general time reversible (GTR) model under assumption of the gamma distribution to accommodate substitution rates at the remaining sites and the starting tree was obtained via neighbor-joining with nearest neighbors. For the Maximum Parsimony analysis (MP), heuristic search method of tree bisection-reconnection (TBR) branch swapping was used. A neighbor-joining (NJ) analysis was calculated from the Kimura two-parameter model (Kimura 1980) with uniform rates as rate among sites (Saitou & Nei 1987). Bootstrap analyses were performed with 1000 resampled datasets using MEGA 6.05 (Tamura et al. 2013).

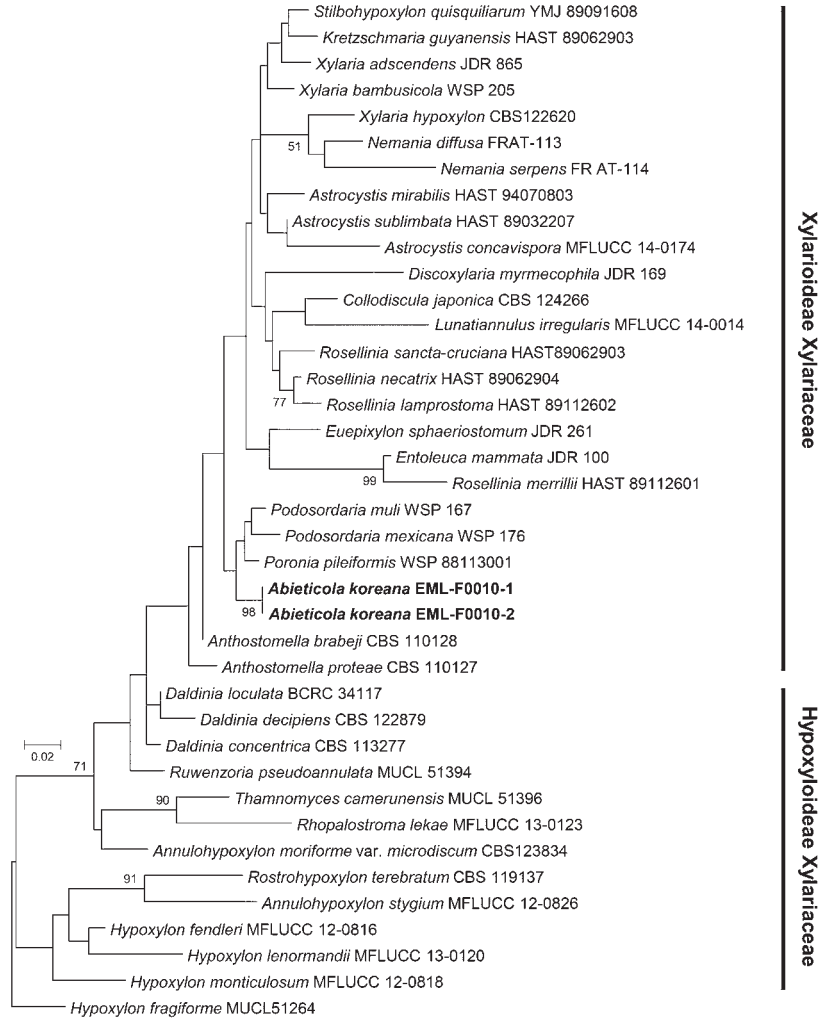


Fig. 1. Phylogenetic position of *Abieticola koreana* EML-F0010-1 and EML-F0010-2 and relationships among *Xylariaceae* based on multigene analysis (ITS, 28S, rpb2, and β-tubulin). *Hypoxylon fragiforme* was used as outgroup. The scale bar represents one substitution per 100 nucleotides. The trees were constructed based on Maximum Likelihood (ML). Phylogenetic classification by Daranagama et al. (2015).

Results

Phylogenetic analysis

F0010-1 and F0010-2 clustered within a clade including *Podosordaria* and *Poronia* species, but formed a separate monophyletic group in maximum-

likelihood (ML) trees. BLASTn search revealed that the ITS sequence of F0010-1 strain (JN977612) was 96.5% identical with *Pod. mexicana* WSP 176 (GU324762; 409 of 424 bp), 97.2% identical with *Pod. muli* WSP 167 (GU324761; 412 of 424 bp), and 96.5% identical with *Por. pileiformis* WSP 88113001 (GU324760; 409 of 424 bp). However, the sequences of F0010-1 were <95% similar to those of several isolates known to be species of *Xylaria* (FJ612908; EU716506; AY315407; EU716509). In addition, the BLASTn search results for the 28S rDNA sequence of strain F0010-1 (JQ014618) was 98.1% homologous with *Xylariaceae* sp. 1175 (FJ425703; 527 of 537 bp), 97.8% homologous with *Xylaria* sp. DIS99a (DQ327620; 715 of 731 bp), and 97.6% homologous with *Xylaria* sp. DIS255j (DQ327627; 721 of 739 bp).

To further elucidate the phylogenetic status of isolate F0010, sequence data for additional genes, rpb2, and β-tubulin were analysed. The BLAST results for the rpb2 and β-tubulin gene sequences indicated that the fungus is somewhat closely related to genera such as *Podosordaria* and *Poronia*, but showing identity values of only 76.6–81.0% with *Pod. mexicana* WSP 176, 83.0–83.9% with *Pod. muli* WSP 167, and 73.2–82.2% with *Por. pileiformis* WSP 88113001. Based on the sequence data and the structure of the conidial anamorph, isolate F0010 appeared to constitute a new genus and species, forming a distinct lineage within the Xylarioideae clade, sister to the *Podosordaria*/*Poronia* lineage (Fig. 1).

Taxonomy

Abieticola Hyang B. Lee, **gen. nov.**

MycoBank MB 811702

Differs from *Poronia* by its slightly curved conidia, and its shorter conidiogenous cells.

Type species: *Abieticola koreana* Hyang B. Lee

Etymology: *Abieticola*, meaning "*Abies*-inhabiting", referring to the host genus.

A coremioid, synnematous conidial anamorph produced on artificial media is characterized by small clubs with white heads consisting of coiled conidiogenous hyphae and synnemata that resemble those of *Poronia* species but differ in bearing slightly curved conidia from shorter conidiogenous cells, some of which bear three conidia.

Abieticola koreana Hyang B. Lee, **sp. nov.** Figs 2, 3

MycoBank MB 811703

Differs from *Poronia* spp. by its slightly curved conidia, and its shorter conidiogenous cells that sometimes bear 3 conidia.

Type: Republic of Korea, Daejeon, Yuseong-gu, endophytic in inner bark of *Abies holophylla* Maxim. (*Pinaceae*), August 2006 (CNUFC EML-F0010-1 [preserved as

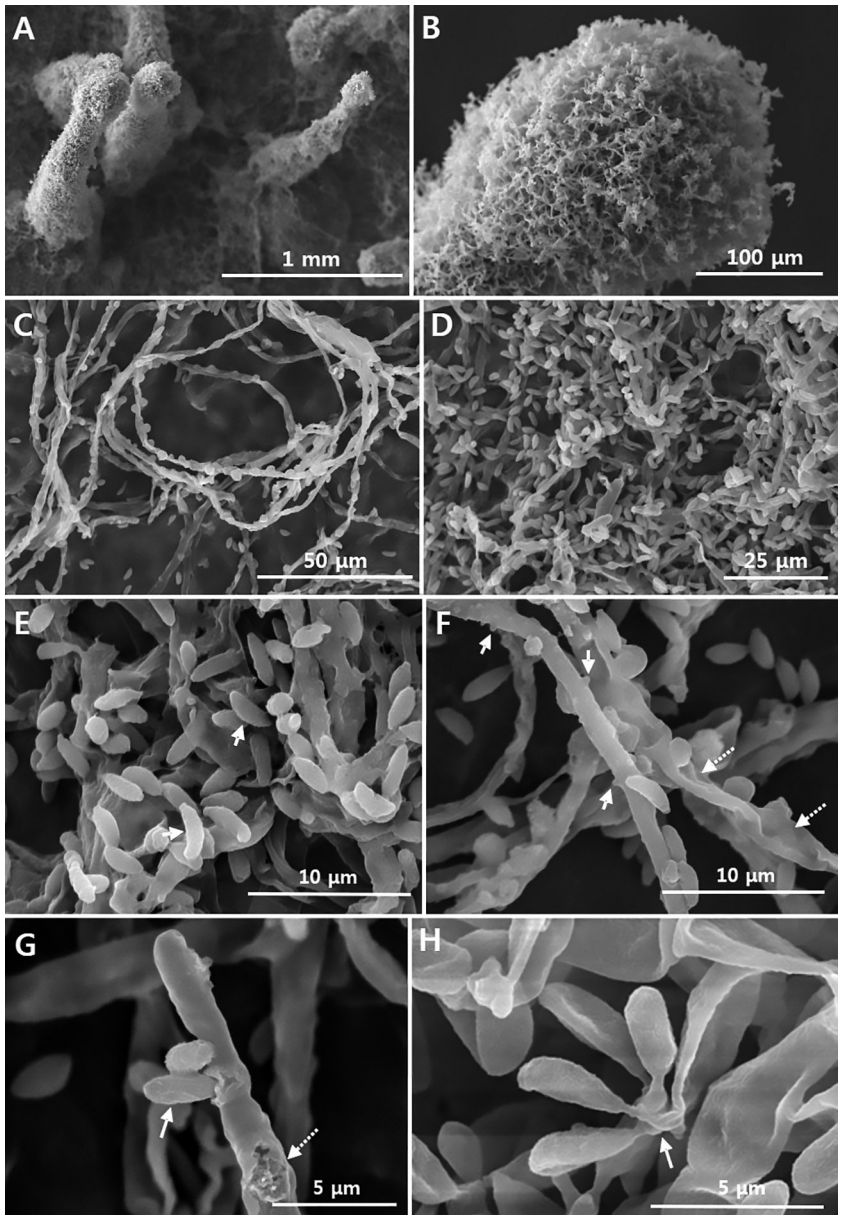
metabolically inactive frozen culture]; ex-type cultures, KCTC 10629BP, CNUFC EML-F0010-1; GenBank JN977612, JQ014618, KP792128, KP792126).

Etymology: *koreana,* referring to the country of isolation, Korea.

Stromata frequently developed on PDA medium, resembling small clubs with white heads, ≤20.5 mm tall and with blackish brown exudates oozing out along the lower stalk. Substrate mycelia forming in abundance, but aerial mycelia poorly produced. Synnemata generally erect but curving in age, 8.0–20.5 (av. 13.15 ± 1.4) × 0.9–1.4 mm, with knotted hyphae of variable thickness (mostly 1–1.8 µm), pigmented brown to yellow in age; functioning as conidiophores. Conidia 1 or 2 (sometimes 3) in number, produced laterally on mycelia and detaching with distinct scars, 2.5–4.0 × 1.0–1.5 (av. 3.0 × 1.5) µm on PDA,

Fig. 2 (above). *Abieticola koreana* (F0010-1): macromorphological culture characteristics. A, B: PDA containing 5% wild dog dung culture at 27°C for 1 month (A) and 2 months (B – plate refrigerated for 2 days and then moved to RT to induce fruiting); C, D: PDA culture at 18°C for 1 month (C) and 50 days (D); E: MEA culture with abundant mycelia and aerial club-like synnemata with long whitish heads and yellowish bases; F: culture on 5% dung medium at 27°C for 50 days; G: culture on PDA containing 5% dung at 27°C for 50 days; H: culture on MEA medium at 27°C for 1 month; I and J: culture on PDA containing 5% dung at 27°C for 50 days; K: old fruit bodies producing whitish powdery conidia and hairy mycelia (yellow arrow) in age on PDA, L: culture on PDA containing 5% dung at 27°C for 2 months (plate refrigerated for 2 days and then moved to RT to induce fruiting).

Fig. 3 (right). *Abieticola koreana* (F0010-1): micromorphological culture characteristics. A: fruit bodies of the vase-shaped structure on PDA; B: magnified picture of the round head shown in A; C: slender, long, tangled thread-like mycelia with conidia, D: magnified picture of numerous conidia



on mycelia shown in C, E: magnified pictures of anamorphic conidia in different forms including elliptical, or oblong to banana-like (solid arrow) shown in C and D, F: conidia and normal mycelia with small knots (solid arrow) and flat mycelia (dotted arrow), G: conidia (solid arrow) and scar (dotted arrow) formed on mycelia, H: conidiation in cluster (solid arrow) on mycelia.

hyaline, bases brown to yellow, tips white to grey; morphologically variable, *Poronia*-like, ellipsoid or fusoid to ovoid, often slightly curved. No teleomorph found on artificial media. Colonies on PDA 25 mm diam. after two weeks at 25°C, yellow to brown in age.

Additional strains: **REPUBLIC OF KOREA**, **Daejeon,** Yuseong-gu, endophytic in inner bark of *Abies holophylla*, August 2006 (CNUFC EML-F0010-2 to F0010-10).

Distribution: Known only from the type locality in South Korea.

Growth

Colonies attained an average diameter of 25 mm on MEA medium after two weeks incubation at 25°C (the optimum temperature for growth). The medium became pigmented yellow to brown with age. Mycelial growth rates were determined under different water activity conditions (0.90–0.999 a_w; Fig. 4. Growth of strain F0010-1 was maximal at 0.999 a_w/25°C, with a similar growth rate at 0.98 a_w, but with almost no mycelial growth at 0.90 a_w, regardless of temperature. At 0.95 a_w, the strain showed moderate growth on medium amended with glucose, but no growth on medium amended with KCl solution. The strain showed almost no mycelial growth at lowered water availability level (0.90 a_w) regardless of temperature.

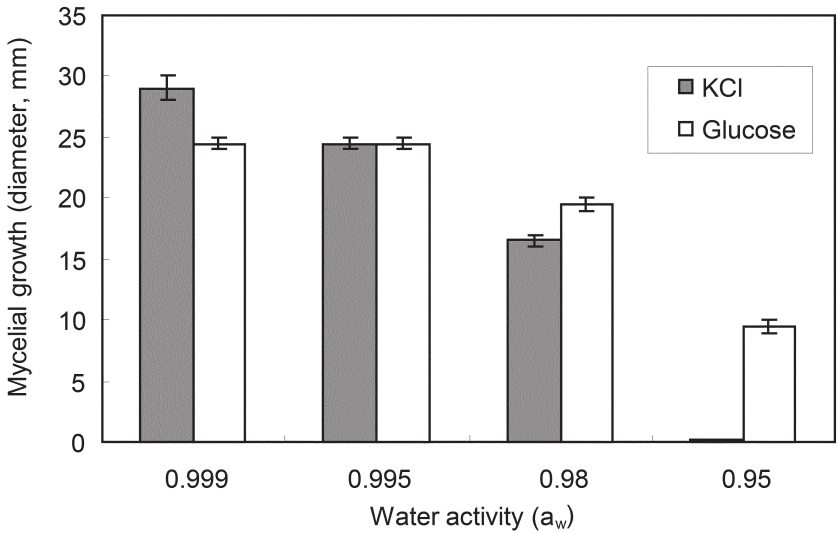


Fig. 4. *Abieticola koreana* (F0010-1): effect of water activity (a_w) on mycelial growth on PDA. The water activity levels were adjusted using two different solutes, KCl (left) and glucose (right).

Discussion

Several attempts were made to induce a teleomorph of *Abieticola koreana* on a variety of artificial media (including dung in some media because some xylariaceous fungi are coprophilous), but our attempts were unsuccessful. The conidiomata that formed in culture on artificial media were tested for germination in solution, but no spores germinated within 3 days.

Anamorphs of *Xylariaceae* differ in their modes of conidiogenesis and the complexity of the conidiophores, and these characters have previously been employed to define different form-genera (Hsieh et al. 2005). No teleomorphic structures were observed here on various artificial media including dung-containing media.

Although many related genera produce anamorphs on developing stromata, few detailed ultrastructural studies of these anamorphs have been published. The anamorphs of genera such as *Calceomyces*, *Daldinia*, *Hypoxylon*, *Rhopalostroma*, *Thamnomyces*, and *Thuemenella* ('Hypoxyloideae') have *Nodulisporium*-like anamorphs (Stadler et al. 2013); and *Xylocoremium flabelliforme* has been reported as the anamorph of *Xylaria cubensis* (Rogers 1984, Hsieh et al. 2005). The ultrastructure of the stromata and conidia and mode of conidiogenesis in *A. koreana* could be used to distinguish it from similar xylariaceous anamorphs. The mode of conidiogenesis on the surface of the synnemata of *A. koreana* was examined by SEM and found similar to other *Xylariaceae* (including those in *Podosordaria*, *Poronia*, and *Xylaria*), but the morphology of the synnemata, conidiomata, and conidia differed from those of the related genera.

Conidia formed by *A. koreana* resembled those of *Pod. leporina*, but differed slightly in that they were produced by geniculate conidiogenous cells; the elongated tips of the synnemata resembled those of *Por. ingii* in being slightly shorter but differed in the ellipsoid and subglobose conidia (Koehn & Cole 1975, Ribes & Negrin 2011). According to Rogers et al. (1998), *Podosordaria* has *Geniculosporium*-like anamorphs, while *Poronia* has *Lindquistia*-like anamorphs. *Podosordaria*, with a typically stalked stroma with a subglobose head, differs from *Poronia*, with a flattened disc (Krug & Cain 1974).

Koehn & Cole (1975) compared the ultrastructure of *Pod. leporina* and *Por. oedipus* suggesting that *Pod. leporina* should be returned to *Poronia*. The morphology and molecular phylogenetic position of *A. koreana* also differed from these two genera.

Podosordaria and *Poronia* are closely related genera containing species that have been considered close relatives of *Xylaria* but differ by their capitate stromata and coprophilous nature (Deepna & Manimohan 2012). Neighbor-joining (NJ) and maximum parsimony (MP) analyses of four DNA loci (ITS,

28S, β-tubulin, rpb2) performed in this study produced tree patterns almost identical to ML (not shown). Three ITS-based phylogenetic analyses of *Xylaria* and related genera proved to be practical for taxonomic determination of intra-specific relationships in highly morphologically variable fungi like the *Xylariaceae* (Spatafora & Blackwell 1993). Nevertheless, studies based on the ITS marker encountered major issues when attempting to determine phylogenetic relationships with other *Xylaria* species because of its highly variable nature (Hsieh et al. 2010). In addition, the number of *Xylaria*-related sequences in GenBank is limited.

Nevertheless, it is very important to note the phylogenetic status and relatedness of *Abieticola* among xylariaceous fungi because until now no intermediate group related to *Poronia* and *Podosordaria* has been reported. Results revealed that *Abieticola* is a clade within the intermediate group on phylogenetic trees such as ML, NJ, and MP.

Most *Xylaria* species ('Xylarioideae') and some segregates like *Sarcocylon* have elongate stromata; interestingly, *Daldinia asphalatum* ('Hypoxyloideae') also produce elongate stromata. The sporulating structures and attachment patterns of *A. koreana* resembled those of *Poronia* spp. (*Lindquistia*-like) and *Xylaria* spp. (*Geniculosporium*-like), but differed from them in having broader conidiogenous cells and slightly curved conidia.

The presence or absence of specific chemicals can, in many cases, be linked with taxonomic position, which may prove invaluable in determining associations between species, species groups, and genera (Whalley & Edwards 1995). The placement of the F0010-1 and F0010-2 strains in the Xylarioideae clade constructed by Daranagama et al. (2015) suggests that other members of the clade should be investigated for ability to produce griseofulvin.

Endophytic fungi are organisms that live inside plant tissue for at least part of their life cycle without causing any disease symptoms (Petrini 1991, Schulz & Boyle 2005). It is interesting that *Xylaria* species isolated from eastern white pine (*Pinus strobus*) needles and lowbush blueberry (*Vaccinium angustifolium*) stems has been reported to produce griseofulvin (Richardson et al. 2014). Liu et al. (2007) reported that an endophytic *Xylaria* sp. isolated from *Ginkgo biloba* had broad antimicrobial activity. Quang & Bach (2008) also reported that ergosta-4,6,8(14),22-tetraen-3-one produced by a *Xylaria* species from Vietnam can inhibit nitric oxide production. Several metabolites, including linoleic acid, linoleic acid methyl ester, and 4-hydroxyscytalone, were isolated from a methanolic extract of another *Xylaria* species (Jang et al. 2009). A xylariaceous coprophilous fungus, *Pod. tulasnei,* was shown to produce tulasnein, a new metabolite with strong antimicrobial activity and weak cytotoxic and phytotoxic activities (Ridderbusch et al. 2004). *Poronia punctata* has been

shown to produce a range of biologically active sesquiterpenes (Poyser et al. 1985, Anderson et al. 1988). Stadler et al. (2014) noted that fungal secondary metabolite profiles can have taxonomic value beyond the species rank and even coincide with phylogenetic data. Kuhnert et al. (2014) indicated that the biosynthetic pathways of diverse compounds derived from fungi have evolved independently during the evolution of fungi. More studies on the molecular phylogeny and ultrastructure of diverse groups belonging to the Xylarioideae clade, which are related to the new genus and species, are needed.

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